

# Reproductive success of passerines exposed to polychlorinated biphenyls through the terrestrial food web of the Kalamazoo River<sup>☆</sup>

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## Abstract

The eastern bluebird (*Sialia sialis*) and the house wren (*Troglodytes aedon*) were identified as ecological receptors of concern due to exposure and potential effects stemming from polychlorinated biphenyl (PCB) contamination in floodplain soils of the Kalamazoo River Superfund Site, Michigan, USA. Measures of population health were compared at a contaminated and a less-contaminated reference location. During this 3-year study, productivity of bluebirds was significantly less at the downstream location than at the reference location. Hatching success, clutch size, and predicted brood size were significantly less in early clutches of house wrens at the more contaminated location than at the upstream reference location, but fledging success was significantly greater at the contaminated location. Studies concurrent to the study presented here reported that concentrations of PCBs in the tissues and diets of the passerine birds were less than the predicted threshold for adverse effects. The results of our study, taken along with the measured exposure data, suggest that other factors in addition to PCB exposure such as habitat, prey availability, small sample size, and cocontaminants were likely causes of the differences that were observed at the two locations.

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## 1. Introduction

The Kalamazoo River was designated a Superfund site in 1990 due to the presence of wastes contaminated with polychlorinated biphenyls (PCBs) that were released during the carbonless copy paper recycling process (MDEQ,

2003). The Kalamazoo River Area of Concern (KRAOC) supports a diverse terrestrial ecosystem along its course, including 205 ha of formerly impounded contaminated sediment exposed when three dams were partially dismantled to their sill levels. While the effects of PCB contamination on various species with aquatic-based diets is well documented (Kubiak et al., 1989; Ludwig et al., 1993; Yamashita et al., 1993; Giesy et al., 1994; Millsap et al., 2004), few studies have evaluated PCB contamination in wildlife exposed through terrestrial food webs associated with riverine ecosystems. The presence of PCBs in soil and sediment at the site requires identification of effects in terrestrial and aquatic endpoints during the risk assessment process. This study focused on the effects of PCB contamination in two passerine birds with predomi-

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nantly terrestrial diets, while risk to passerines dependent on the aquatic food web is presented elsewhere (Neigh et al., 2006a).

There is a well-established history for the use of passerines as biosentinels of contamination by scientists at the US Environmental Protection Agency (USEPA) (Ankley et al., 1993), Environment Canada (Bishop et al., 1995), the Canadian Wildlife Service (Elliott and Harris, 2002), and the US Fish and Wildlife Service (USFWS) (McCarty and Secord, 1999), but studies frequently focused on a receptor of the aquatic food web, the tree swallow. Presently, there is no such favored passerine receptor that exists for terrestrial systems, although in terrestrial risk assessments, depending on habitat type and species applicability to site-specific monitoring criteria, a number of passerine species have been utilized. These species include the European starling (*Sturnus vulgaris*) (Halbrook et al., 1998), American robin (*Turdus migratorius*) (Henning et al., 1997, 2002), red-winged blackbird (*Agelaius phoeniceus*) (Bishop et al., 1995), western bluebird (*Sialia mexicana*) (Fair and Myers, 2002), ash-throated flycatcher (*Myiarchus cinerascens*) (Fair and Myers, 2002), American redstart (*Setophaga ruticilla*), barn swallow (*Hirundo rustica*), eastern phoebe (*Sayornis phoebe*), rose-breasted grosbeak (*Pheucticus ludovicianus*), wood thrush (*Hylocichla mustelina*), and yellow warbler (*Dendroica petechia*) (Henning et al., 1997). To our knowledge, eastern bluebirds (*Sialia sialis*) have been used infrequently as a reproductive monitor of exposure (Bishop et al., 1995), and the house wren (*Troglodytes aedon*) is a novel monitor of terrestrial PCB exposure. In addition, there is a lack of field and laboratory toxicity studies on these species even though they are fairly ubiquitous, abundant, and easily captured contaminant receptors. There are also a large number of studies in the current literature investigating breeding, behavior, and other aspects of biology at relatively uncontaminated ecosystems from which to establish baseline values for comparison to contaminated locals. These two species are often listed as species of concern at sites contaminated with PCBs, so, to fill the data gap in the current literature, this part reports empirical information on the responses of these species to the potential effects of PCBs under field conditions.

The house wren and eastern bluebird were selected as monitors of PCB exposure at the Kalamazoo River Superfund site based on a number of criteria but primarily because they were named as resident species of concern due to their exposure to contaminated soils through the diet (MDEQ, 2003). They were not chosen as surrogate species or sensitive sentinels for other species but rather were studied to determine the potential for exposure of these species and to determine ecologically relevant reproductive parameters. Eastern bluebirds and house wrens frequently used nest boxes at the Kalamazoo River locations, so sample sizes were expected to be sufficient to demonstrate population health. It was also thought that the exposure of this species to PCBs would coincide with local sources of

contamination in the soil due to their diet of terrestrial insects (Pinkowski, 1978), and thus be useful to assess potential reproductive impairment by PCBs. The eastern bluebird was also identified as a species of concern due to plant-derived sources of PCB exposure in its diet (MDEQ, 2003). Here, we describe productivity and growth of house wrens and eastern bluebirds located within the Kalamazoo River Superfund site and an upstream reference site.

## 2. Materials and methods

### 2.1. Site details

During 2000, nest boxes were established at two locations along the Kalamazoo River's 100-year floodplain. The Fort Custer State Recreation Area (FC) is located upstream of the sources of contamination and therefore was chosen to serve as a background location due to the low background levels of contamination existing at the site (Anonymous, 1997). Situated 67 km downstream, the target area was located at the former Trowbridge Impoundment (TB) within the KRAOC, which was designated a Superfund site by the USEPA in 1986 (Fig. 1). The former TB was formed when the Trowbridge Dam was removed to the sill, which exposed 132 ha of former depositional lake bottom sediments.

Previous studies quantified concentrations of total PCBs, dichlorodiphenyltrichloroethane (DDT) isomers, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) (Neigh et al., 2006b) based on World Health Organization toxic equivalence factors (TEF<sub>WHO-Avian</sub>) (van den Berg et al., 1998) in the diet, eggs, nestlings, and adults of eastern bluebirds and house wrens. This study was designed as a comparative study between two different locations; therefore, concentrations in eggs were not converted to fresh weight values. Eggs were sampled at similar times and stored for similar periods of time, so moisture content was expected to be similar in sampled eggs at the two sites. In all tissues from both eastern bluebirds and house wrens, mean concentrations of total PCBs and TEQs were significantly greater at the KRAOC than they were at the reference location, FC (Table 1). Among all matrices, concentrations of total PCBs at TB were 39- to 122-fold greater than concentrations at FC, while concentrations of TEQs at TB were 5- to 47-fold greater than concentrations at FC. Although not significantly greater, concentrations of DDT isomers in eastern bluebird eggs were 1.4-fold greater at TB than at FC, and concentrations of DDT isomers in house wren eggs were 3.3-fold greater at TB than at FC. Concentrations of total PCBs moderately bioaccumulated from lower trophic level species to upper trophic level predators, but concentrations in biota were less than those in soils. The decrease in the concentrations of total PCBs from soil to biota was accompanied by a decrease in relative potency (mg TEQ/kg PCB) of the PCB mixture, which was attributed to environmental weathering (Blankenship et al., 2005).

### 2.2. Productivity observations

Throughout the 2001–2003 nesting seasons, nest boxes were monitored for occupancy by eastern bluebirds and house wrens at FC ( $n = 64$ ) and TB ( $n = 68$ ). Boxes were monitored daily to determine the date of clutch initiation and day of hatch. Reproductive success was determined based on nest observations. Hatching success was described as the percentage of eggs hatched in each nest. Fledging success was described as the percentage of nestlings fledged per nestling hatched, and productivity was described as the percentage of nestlings fledged per egg laid. Egg mass and length was recorded within 24 h of laying. In an effort to minimize the accidental breakage of eggs in the field due to measurement, egg width and therefore egg volume were not investigated. Day of hatch was also confirmed by assessing physical development of the nestling (Pinkowski, 1975). Each nestling was examined for gross external morphological abnormalities. Weight of each nestling was recorded on days 3, 9, and 12

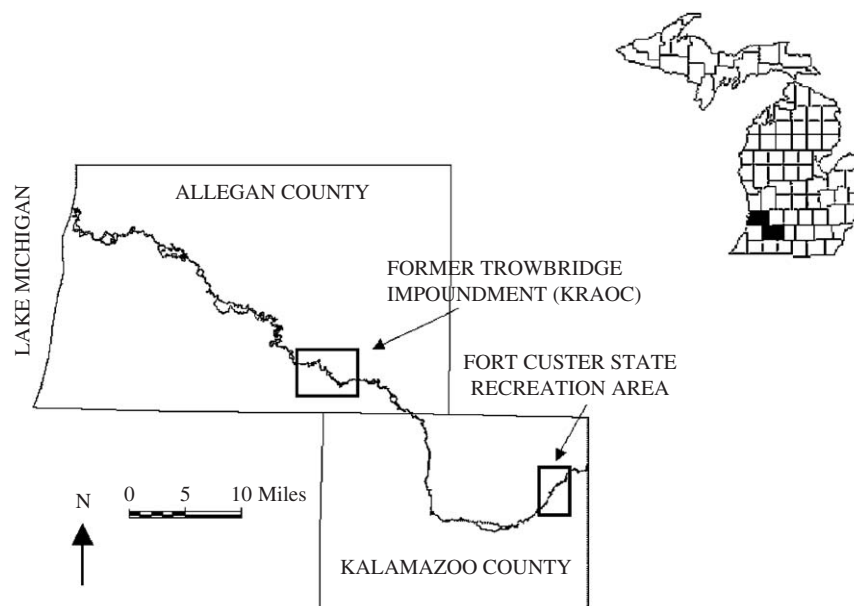


Fig. 1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset shows the location of the two counties (black) in Michigan where reproductive studies were conducted (Neigh et al., 2006a). The boxes designate the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge Impoundment, located within the KRAOC.

Table 1

Mean ( $\pm$ SD) concentrations (wet weight) of total PCBs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), and DDT in the tissues of terrestrial passerine species at the Fort Custer State Recreation Area (FC) and at the Trowbridge Impoundment (TB) (Neigh et al., 2006b)

	Total PCBs (mg PCB/kg)				TEQs <sup>a</sup> ( $\mu$ g TEQ/kg)				DDT <sup>b</sup> (mg DDT/kg)			
	<i>n</i>	FC	<i>n</i>	TB	<i>n</i>	FC	<i>n</i>	TB	<i>n</i>	FC	<i>n</i>	TB
<i>Eastern bluebird</i>												
Egg	14	0.17 (0.10)	7	8.3 (5.1) <sup>c</sup>	3	7.6 (8.6)	5	77 (82) <sup>c</sup>	5	1.5 (1.8)	5	2.1 (1.1)
Nestling	17	0.011 (0.006)	6	1.3 (1.4) <sup>c</sup>	17	1.3 (0.64)	6	6.3 (4.0) <sup>c</sup>	5	NA	5	NA
<i>House wren</i>												
Egg	14	0.12 (0.12)	14	6.3 (6.0) <sup>c</sup>	8	8.6 (3.9)	11	400 (450) <sup>c</sup>	5	0.66 (0.87)	5	0.20 (0.21)
Nestling	13	0.020 (0.020)	17	0.77 (0.64) <sup>c</sup>	13	1.4 (0.96)	17	63 (47) <sup>c</sup>		NA		NA
Adult	8	0.072 (0.033)	9	3.2 (2.1) <sup>c</sup>	8	7.1 (5.7)	9	110 (57) <sup>c</sup>		NA		NA

<sup>a</sup>TEQs based on World Health Organization Toxic Equivalence Factors (van den Berg et al., 1998).

<sup>b</sup>DDT includes *o*, *p'* and *p*, *p'* isomers of DDT, DDD, and DDE.

<sup>c</sup>Mean is significantly greater than that at the FC reference area (Students *t* test,  $P < 0.05$ ).

(hatch day = day 0) for eastern bluebirds and on days 3, 6, and 9 for house wrens. When chicks were sampled for contaminant analysis at approximately day 9 for house wrens and day 12 for eastern bluebirds, each individual was weighed and the length of the entire body (tip of beak to longest rectrix), tarsus (tibiotarsal joint to hind toe base), and unflattened wing chord were recorded. Measurements were taken during 2001 and 2002 for house wrens and from 2001 to 2003 for eastern bluebirds.

### 2.3. Statistical analysis

For statistical analyses (SYSTAT, Evanston, IL, USA), each nest box was treated as a separate experimental unit and values were reported on a per box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with Kolmogorov–Smirnov's one-sample test with Lilliefors transformation, and homogeneity of variance was verified by *F* test. All parametric data were analyzed by one-way analysis of variance (ANOVA), and non parametric data were analyzed by

Mann–Whitney *U* or Kruskal–Wallis tests. The criterion for significance used in all tests was  $P < 0.05$ . Statistical comparisons were made between means and distributions at the two locations. In addition, the relationships between concentrations of PCBs, measured as both total PCBs and TEQs, and specific endpoints were examined by both Spearman and the Pearson correlations.

## 3. Results

### 3.1. Reproductive success

A total of 34 house wren and 18 eastern bluebird nests were completed at the TB study site and 71 house wren and 57 eastern bluebird nests were completed at the FC site over the study period (Table 2). For the entire study, 32% of eastern bluebird nests failed and 24% of house wren

Table 2

Nest fate and percentage of initiated nests of eastern bluebirds and house wrens at the Fort Custer State Recreation Area (FC) reference site and at the Trowbridge (TB) contaminated site

	2001		2002		2003	
	FC	TB	FC	TB	FC	TB
<i>Eastern bluebird</i>						
Successful <sup>a</sup>	14 (93%)	2 (40%)	15 (63%)	2 (25%)	14 (78%)	4 (80%)
Predated <sup>b</sup>	1 (7%)	0 (0%)	6 (25%)	4 (50%)	1 (6.5%)	1 (20%)
Abandoned <sup>c</sup>	0 (0%)	1 (20%)	2 (8%)	2 (25%)	2 (11%)	0 (0%)
Unknown	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	0 (0%)	2 (40%)	1 (4%)	0 (0%)	1 (6.5%)	0 (0%)
Total	15	5	24	8	18	5
<i>House wren</i>						
Successful <sup>a</sup>	17 (89.5%)	8 (73%)	38 (73%)	17 (74%)		
Predated <sup>b</sup>	1 (5.25%)	0 (0%)	6 (11%)	2 (9%)		
Abandoned <sup>c</sup>	0 (0%)	2 (18%)	0 (0%)	3 (13%)		
Unknown	0 (0%)	1 (9%)	5 (10%)	0 (0%)		
Other	1 (5.25%)	0 (0%)	3 (6%)	1 (4%)		
Total	19	11	52	23		

<sup>a</sup>Successful nests fledged at least one nestling.

<sup>b</sup>Predated nests included those nests with evidence of predation such as disturbed nesting material or broken eggs.

<sup>c</sup>Nests were considered abandoned when eggs were cold for at least 7 days and adults were not present.

nests failed. The failure of the majority of eastern bluebird nests was attributed to predation, which comprised over 50% of the nest failures at both FC and TB. Abandonment accounted for 29% of the nest failures at FC and 30% at TB. Failure of house wren nests was attributed to a greater proportion of abandoned nests at TB (56% of all failures), while no nest failures were attributed to abandonment at FC. Predation accounted for 44% of house wren nest failures at FC but only 22% at TB.

Both house wrens and eastern bluebirds can produce two broods in a season. Birds were not banded at either location, and therefore it was not possible to definitively determine when a nesting pair established subsequent nests. In all years, there were 7 and 12 suspected house wren second broods at TB and FC, respectively, and 3 and 15 suspected eastern bluebird second broods at TB and FC, respectively. Nests were still considered to be independent samples because second broods could not be verified since adults were not banded. To limit the effect of second nestings, reproductive parameters were evaluated for the entire nesting season and based on dates of clutch initiation, where nests from early in the season were grouped together and nests from late in the season were grouped together. Nesting attempts were categorized as early or late nests to account for potential differences in reproductive parameters between the first and the second nestings. For example, the productivity of second nesting attempts in eastern bluebirds was about 21% (Pinkowski, 1979). The median dates of initiation for house wrens in 2001 were May 20 and June 24 for early and late nests, respectively, and May 28 and July 1 during 2002, respectively. The median date of nest initiation for eastern bluebirds was between April 21 and 25 for early nests and

between June 9 and 22 for late nests during the study. There was little correlation (Spearman,  $r^2 < 0.10$ ) between date of nesting and reproductive success in either house wrens or eastern bluebirds.

All measures of reproductive success, including hatching success, fledging success, and productivity, were adjusted for fresh egg sampling, except clutch size, by eliminating the egg collected for residue analyses from the reproductive analyses because the subsequent potential fate of the eggs collected for residue analyses could not be predicted at the time of sampling. Nests were observed for activity until the date of fledging, so it was assumed that live sampled nestlings would have fledged successfully. Therefore, the number of nestlings sampled did not affect the outcome of statistical analyses, but the number of fresh eggs sampled could have affected the observed brood size and number of fledglings. A predicted value based on other parameters was used to evaluate brood size and the number of fledglings. The brood size for each nest in which an egg was sampled was calculated as the clutch size multiplied by the hatching success. The predicted number of fledglings for each nest in which an egg was sampled was calculated as the clutch size multiplied by the productivity. All other interactions attributed to the collection of eggs or nestlings were considered to be negligible as compared to exposure to PCBs. The study design, which requires at least one egg or nestling to be sampled from each nest for residue analysis, may limit the compatibility of this study to the published literature, but the main focus of the study was aimed at comparing reproductive success of an unexposed versus an exposed population.

Owing to relatively small sample sizes within a year, measurements of reproductive health in eastern bluebirds

were combined among all years. When the results for all 3 years were combined, a statistically significant difference between FC and TB in productivity (Mann–Whitney *U*) and nearly statistically significant differences ( $P < 0.1$ ) in hatching success (Mann–Whitney *U*) and fledging success (Mann–Whitney *U*) were observed (Table 3). Statistically significant differences were detected in the date of clutch initiation, so, to eliminate interactions between date of initiation and reproductive success, all nests were separated into one of two groups, which were designated “early” or “late” nests. Reproductive success was not statistically different in late nests, but the clutch size (Mann–Whitney *U*) and fledging success (Mann–Whitney *U*) were statistically greater at FC than at TB in early nests.

Reproductive parameters for house wrens were also not statistically different between years, so all years were combined. When all years were combined, the only statistically significant difference between locations was fledging success, which was greater at TB than at FC (Mann–Whitney *U*) (Table 3). Although the date of clutch initiation for house wrens was not significantly different between locations, clutches were also separated into early and late nests. In early clutches, clutch size (Mann–Whitney *U*), hatching success (Mann–Whitney *U*), and predicted brood size (Mann–Whitney *U*) were statistically greater for nests at FC than at TB.

### 3.2. Egg measurements

No statistically significant differences in egg parameters among years were detected, so the results from all 3 years were combined. The mean weights of eggs of neither species were statistically different between locations (Table 4). The mean length of house wren eggs was not statistically different between sites, but eggs were significantly longer at FC than at TB for eastern bluebird eggs (Student's *t* test). Egg parameters were evaluated for interaction with each other and with date of initiation based on ANCOVA. No interactions between any parameters existed for house wren, but a statistically significant correlation between weight and length did exist for eastern bluebirds (ANCOVA), which changed the significance of the comparison of mean egg length ( $P < 0.05$  to  $P > 0.05$ ).

### 3.3. Growth measurements and growth curves

Differences in growth parameters between years could not be evaluated due to small sample sizes, so all years were combined. Growth parameters in 8- and 9-day-old house wrens were statistically similar, so ages were combined. Eastern bluebird growth measurements consisted of nestlings at day 12. Correlations between growth parameters within locations were first taken into account when growth

Table 3

Eastern bluebird and house wren nest productivity measurements (mean  $\pm$  SD) at a reference site (Fort Custer) and at a PCB-contaminated target site (Trowbridge) on the Kalamazoo River for early and late clutches and all clutches combined

	Early clutches				Late clutches				All clutches			
	Fort Custer		Trowbridge		Fort Custer		Trowbridge		Fort Custer		Trowbridge	
	<i>n</i>	Mean ( $\pm 1$ SD)	<i>n</i>	Mean ( $\pm 1$ SD)	<i>n</i>	Mean ( $\pm 1$ SD)	<i>n</i>	Mean ( $\pm 1$ SD)	<i>n</i>	Mean ( $\pm 1$ SD)	<i>n</i>	Mean ( $\pm 1$ SD)
<i>Eastern bluebird</i>												
Hatching success <sup>a</sup>	25	0.82 (0.27)	10	0.61 (0.44)	24	0.75 (0.34)	4	0.54 (0.42)	49	0.79 (0.31)	14	0.59 (0.42)
Fledging success <sup>b</sup>	24	0.96 (0.20)	6	0.75 <sup>c</sup> (0.42)	21	0.95 (0.22)	3	1.0 (0.0)	45	0.96 (0.21)	9	0.83 (0.35)
Productivity <sup>d</sup>	25	0.79 (0.32)	9	0.44 (0.47)	24	0.73 (0.38)	4	0.54 (0.42)	49	0.76 (0.34)	13	0.47 <sup>c</sup> (0.44)
Clutch size	26	4.7 (0.47)	11	3.5 <sup>c</sup> (1.4)	31	3.6 (0.93)	7	3.7 (1.7)	57	4.2 (1.0)	18	3.6 (1.5)
Predicted brood size	24	4.1 (1.2)	7	3.5 (1.2)	21	3.6 (0.93)	3	2.9 (2.0)	45	3.8 (1.1)	10	3.3 (1.4)
Predicted number of fledglings	23	4.1 (1.2)	5	3.1 (1.5)	20	3.6 (0.94)	3	2.9 (2.0)	43	3.9 (1.1)	8	3.0 (1.6)
<i>House wren</i>												
Hatching success <sup>a</sup>	32	0.83 (0.24)	15	0.58 <sup>c</sup> (0.39)	28	0.78 (0.25)	16	0.71 (0.44)	60	0.81 (0.25)	31	0.64 (0.41)
Fledging success <sup>b</sup>	31	0.90 (0.26)	13	1.0 (0.0)	28	0.94 (0.21)	12	1.0 (0.0)	59	0.92 (0.24)	25	1.0 <sup>c</sup> (0.0)
Productivity <sup>d</sup>	32	0.75 (0.30)	15	0.58 (0.39)	28	0.73 (0.30)	16	0.71 (0.44)	60	0.74 (0.30)	31	0.64 (0.41)
Clutch size	38	6.2 (0.93)	15	5.6 <sup>c</sup> (1.2)	33	5.3 (1.2)	21	5.2 (1.5)	71	5.7 (1.1)	36	5.4 (1.4)
Predicted brood size	31	5.5 (1.5)	13	3.9 <sup>c</sup> (2.2)	28	4.4 (1.6)	12	5.4 (1.3)	59	5.0 (1.6)	25	4.6 (2.0)
Predicted number of fledglings	29	5.2 (1.4)	13	3.9 (2.2)	27	4.2 (1.6)	12	5.4 (1.3)	56	4.8 (1.6)	25	4.6 (2.0)

<sup>a</sup>Hatching success is calculated as the number of eggs hatched per egg laid.

<sup>b</sup>Fledging success is calculated as the number of fledglings per nestling hatched.

<sup>c</sup>Mean of the Trowbridge population is statistically different from Fort Custer population ( $P < 0.05$ ).

<sup>d</sup>Productivity is calculated as the number of fledglings per egg laid.

Table 4

Mean ( $\pm 1$  SD)<sup>a</sup> reproductive endpoints for eastern bluebird and house wren eggs and nestlings reared at the Fort Custer State Recreation Area and at the Trowbridge Impoundment along the Kalamazoo River Area of Concern

	Fort Custer		Trowbridge	
	Eastern bluebird	House wren	Eastern bluebird	House wren
<i>Eggs</i>				
<i>n</i>	36	74	11	37
Egg weight (g)	3.22 (0.67)	1.46 (0.01)	3.01 (0.12)	1.51 (0.09)
<i>n</i>	34	74	11	37
Egg length (mm)	21.4 (1.04)	16.9 (0.08)	20.6 (0.28) <sup>b</sup>	16.8 (0.16)
<i>Nestlings<sup>c</sup></i>				
<i>n</i>	12	22	3	12
Body weight (g)	27.79 (2.04)	9.43 (0.81)	29.17 (2.03)	9.32 (1.39)
Body length (mm)	92.3 (6.2)	64.9 (6.2)	98.1 (14.6)	61.5 (4.2)
Tarsal length (mm)	19.3 (1.8)	16.8 (2.7)	19.7 (1.4)	16.2 (2.4)
Wing chord (mm)	49.9 (8.5)	23.1 (4.2)	47.9 (4.9)	24.4 (4.6)

<sup>a</sup>Mean values were calculated based on the mean measurements per nest for all nests.

<sup>b</sup>Statistically different from Fort Custer population (Students *t* test,  $P < 0.05$ ).

<sup>c</sup>Eastern bluebird nestling measurements were taken at day 12 and house wren nestling measurements at day 8 or 9.

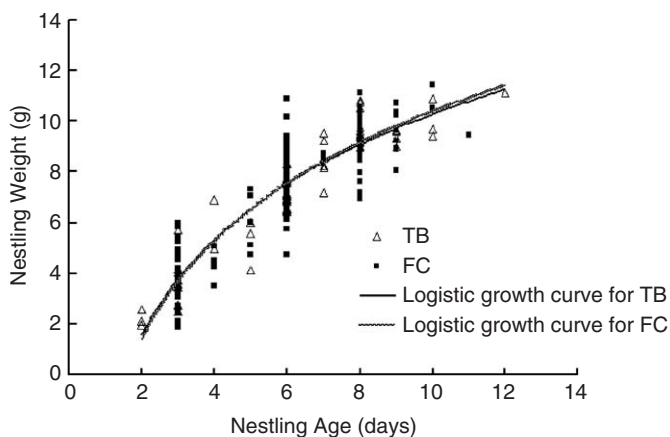


Fig. 2. House wren nestling growth curves based on mean nestling weights for each nest box at the Trowbridge (TB) target area and the Fort Custer (FC) reference site. The equation for the line of best fit at FC is  $y = 5.563 \ln(x) - 2.4223$ ,  $r^2 = 0.836$ . The line of best fit for TB is  $y = 5.378 \ln(x) - 2.1186$ ,  $r^2 = 0.896$ .

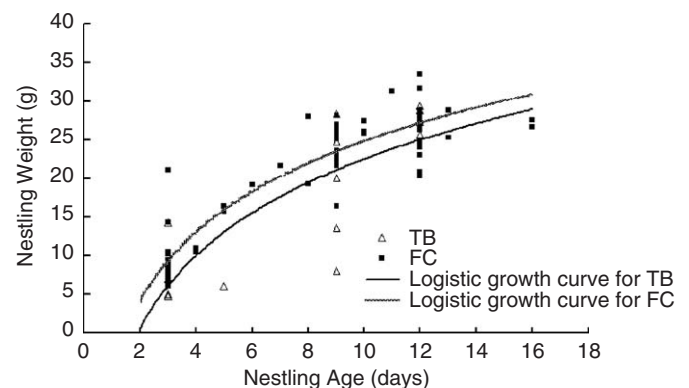


Fig. 3. Eastern bluebird nestling growth curve based on mean nestling weights in each active nest box at the Trowbridge (TB) target site and the Fort Custer (FC) reference site. The line of best fit for the curve is described by the equation  $y = 13.668 \ln(x) - 8.8892$ ,  $r^2 = 0.647$  at TB and  $y = 12.891 \ln(x) - 4.7993$ ,  $r^2 = 0.861$ .

was evaluated between locations. There were no significant correlations present for eastern bluebirds, but there were several potential correlations for house wrens. The correlation between body length, wing chord length, and tarsal length drove the difference in mean body length between sites to a nearly statistically significant value (ANCOVA,  $P < 0.1$ ), but no growth parameters for either species were statistically different between sites (Table 4).

Growth of nestlings was compared between sites based on curves generated from nestling mass gained per day as an indicator of stress on the species (Zach and Mayoh, 1982). A logistic growth curve was fitted to nestling masses at each site. Growth curves, based on average nestling mass for each box at each nestling day, were strikingly similar for both species at both sites when all years were combined.

Growth curves of house wrens, compared between sites, overlapped over the entire nesting period (Fig. 2). Growth curves for eastern bluebirds followed a similar trajectory, but the curve was shifted toward greater mean nestling weights at FC (Fig. 3).

Growth curves, based on average nestling mass gain over the nestling period for the two sites, were compared statistically. Nestling mass between nestling days 3 and 10 for eastern bluebirds and between nestling days 2 and 8 for house wrens was log-transformed and the mass gained per day of life was compared between sites. House wren mass gain per day was significantly different between years (ANOVA), but that of eastern bluebirds was not. House wren mass gain was significantly less at TB than at FC during 2002 (Students *t* test) but not during 2001. Eastern

bluebird mass gain per day was not different between sites in 2001 or 2003, but in 2002 it was significantly different (Kruskal–Wallis).

#### 4. Discussion

##### 4.1. Reproductive success

Eastern bluebirds made fewer nesting attempts than house wrens at TB and FC, but confounding factors such as vegetation structure and age of the nest trail may contribute to this difference (Munro and Rounds, 1985). The bluebird box trail at FC has been established for over 30 years, which may cause an increase in the number of returning adults and nestlings to the site.

Abandonment rates for tree swallows have been reported to be greater at PCB-contaminated sites (5.95–29.5 mg PCB/kg, mean concentrations in eggs, wet weight (ww)) compared to a less-contaminated habitat (0.103 mg PCB/kg, mean concentration in eggs, ww) (McCarty and Secord, 1999). Few data for the bluebird or the house wren are available for abandonment rates at PCB-contaminated sites. Abandonment rates of eastern bluebirds and house wrens at the Kalamazoo River were between 0% and 25%, which was near the range (4.5–23.7%) reported for uncontaminated populations of bluebirds (Rustad, 1972). Abandonment rates of eastern bluebirds may be partly attributed to interactions with house wrens as indicated by observations of house wrens preventing eastern bluebirds from entering the nest or returning with prey items for the nestlings and frequent squabbling between the two species at nest locations. House wrens are known to interfere with the nesting of other species (Finch, 1990) and, unlike other studies of this type, house wren nesting was not discouraged. Abandoned nests had no physical damage to the structure of the nest, as is the case in 85% of house wren predations (Belles-Isles and Picman, 1986), so abandoned nests may have been improperly labeled as abandoned rather than associated with house wren interference. Abandonment was not observed for house wrens at FC, but some nests were abandoned at TB. Again, interactions among house wrens may have caused abandonment without damage to the nest, where in some cases as much as 89% of unsuccessful nesting attempts have been linked to conspecific interference (Finch, 1990).

In an effort to further characterize interactions between eastern bluebird and house wren populations, the correspondence of house wren presence and nesting failure was investigated for eastern bluebird nests. A simple nearest-neighbor-type analysis was conducted to determine whether eastern bluebird failed more often when the nearest nest box was occupied by a house wren or whether a house wren nested in the box immediately following predation or abandonment of the eastern bluebird nest (Table 5). For eastern bluebird nests, 78% of nest predation and 40% of nest abandonments were associated with house wren presence, whereas only 21% of successful

nests were associated with house wren presence. These values suggest that there is an association between presence of house wrens in the temporal and proximal vicinity of eastern bluebird nests and success of eastern bluebird nests.

The date of clutch initiation did not affect the reproductive success of either house wrens or eastern bluebirds. Some studies have reported that second clutches were not as likely to be successful as first clutches, with only 21% of second nests producing fledglings (Pinkowski, 1979). At the Kalamazoo River, there was no reduction in nest success in the clutches classified as “late” nests, but which nests were in fact second nests and which nests were late first nest attempts could not be determined. Other studies also did not find a correlation between Julian date and reproductive parameters (Fair and Myers, 2002).

PCBs may affect reproductive capacity by decreasing hatching success, possibly by causing infertility of eggs (McCarty and Secord, 1999). Hatching success in early clutches of house wrens was significantly less at TB than at FC (Mann–Whitney  $U$ ,  $P = 0.045$ ), which was also observed in tree swallows from the Hudson River where hatching success was 64–79% at contaminated sites (11.7–42.1 mg PCB/kg, mean concentrations in eggs, ww) and 93% at a reference location (0.103 mg PCB/kg, mean concentrations in eggs, ww) (Secord and McCarty, 1997). Conversely, hatching success (87.4%) was not impaired at the Housatonic River for several species of passerines exposed to concentrations of PCBs in floodplain soils (mean concentrations in soils = 12 mg PCB/kg, dry weight, (dw)) (Henning et al., 1997), similar to those at TB (11 mg PCB/kg, dw). Of the nonviable eggs at the Hudson River, 60% were infertile, while the remaining nonviable egg mortality was attributed to the failure of the embryo to develop and the death of the embryo (McCarty and Secord, 1999). Hatching success of both the bluebird and the house wren at the Kalamazoo River are most strongly correlated with productivity (Spearman,  $r^2 = 0.78$ ). Productivity was significantly less in the eastern bluebird at TB, but this value is dependent on a single female in 2001 that twice attempted to produce a brood without success but did not abandon the eggs. This one female alone accounted for a 40% decrease in reproductive success at TB because only five nests were established by bluebirds during that year. If this one individual was removed from the data set, the hatching success at TB for all nests would increase by 10% and the productivity would increase by 9%. Productivity for all nests was no longer significantly different between locations when the nest was removed from the analyses, but all other statistical comparisons were similar to those made previously. Based on clutch size, hatching success, and productivity, reproductive performance was apparently depressed at TB compared to FC, but measured concentrations of PCBs in the tissue and diet of both species were below the threshold concentrations for effects (Neigh et al., 2006b,c). Little risk of reproductive effects was predicted from concentrations of PCBs in tissues and diet. Thus, the lesser reproductive success observed in these

Table 5  
Correspondence of eastern bluebird nest fate<sup>a</sup> to proximal and temporal presence of house wrens

	House wren		Other bird species		Empty nest	
	Nearest neighbor	Later nest	Nearest neighbor	Later nest	Nearest neighbor	Later nest
<i>Eastern bluebird</i>						
Successful (33)	5 (15%)	2 (6%)	16 (49%)	9 (27%)	12 (36%)	22 (67%)
Predated (9)	3 (33%)	4 (45%)	1 (11%)	3 (33%)	5 (56%)	2 (22%)
Abandoned (5)	1 (20%)	1 (20%)	1 (20%)	3 (60%)	3 (60%)	1 (20%)
Other (3)	0 (0%)	1 (33%)	1 (33%)	1 (33%)	2 (67%)	1 (33%)
Total (50)	9 (18%)	8 (16%)	19 (38%)	16 (32%)	22 (44%)	26 (52%)

<sup>a</sup>Two eastern bluebird nests known to be predated by raccoons were removed from the analysis.

Table 6  
Power ( $1-\beta$ ) to detect a decrease in reproductive success at Trowbridge compared to that at Fort Custer and the sample size ( $n$ ) needed at each location to detect a 20% reduction in reproductive health

	Eastern bluebird						House wren					
	Separate clutches				All clutches		Separate clutches				All clutches	
	Early clutches		Late clutches		$1-\beta$	$n$	Early clutches		Late clutches		$1-\beta$	$n$
	$1-\beta$	$n$	$1-\beta$	$n$			$1-\beta$	$n$	$1-\beta$	$n$		
Hatching success	0.29	63	0.16	80	0.38	68	0.63	46	0.09	67	0.56	56
Fledging success	0.22	37	0.18	9	0.19	29	0.57	14	0.33	8	0.73	11
Productivity	0.54	81	0.14	92	0.60	84	0.32	67	0.05	83	0.22	74
Clutch size	0.79	15	0.05	45	0.36	28	0.41	10	0.06	20	0.20	15
Predicted brood size	0.21	26	0.09	60	0.19	33	0.67	35	0.55	35	0.14	42
Predicted number of fledglings	0.29	35	0.09	59	0.33	39	0.50	41	0.70	38	0.07	45

birds was most likely the result of other factors present in the environment. The habitat at the more contaminated location was also deemed to be poor habitat due to frequent flooding and the presence of a dense cover of chord grass.

Statistical power to detect differences in reproductive measurements at the different locations was generally less than an acceptable limit for both species ( $1-\beta < 0.80$ ) (Table 6). Sample sizes greater than those observed at the Kalamazoo River would have been needed to detect a 20% decrease in reproductive performance at TB compared to FC. Unlike laboratory studies where sample sizes can be adjusted based on statistical power analyses to allow differences of a particular magnitude, it is not possible to increase the number of nests in field studies beyond the carrying capacity of the site. At the Kalamazoo River, nest boxes were placed to maximize occupancy and not affect survivorship. Therefore, the limiting factor for the number of nest boxes was the size of the study area, which was beyond the control of the investigators. Although some uncertainty may be associated with measures of reproductive performance due to small sample sizes, there is inherently less uncertainty in the evaluation of reproductive performance and associated exposure levels in the

Kalamazoo River populations of eastern bluebirds and house wrens that were actually measured than to uncertainties associated with predicting responses in these receptors of concern based on distantly related surrogate laboratory species.

The fledging success and growth of nestlings, which may be related to nestling survival, has been associated with proximity to contamination (Fair et al., 2003). Fledging success and growth over the entire study was not impaired in house wrens at TB compared to FC. In eastern bluebird nests, fledging success was less at TB in early nests but not for all clutches combined, and growth over the entire study was not different. Although growth for both species was different in 1 year but not over the entire study, the recruitment of the population, described as the predicted number of fledglings, was not different. The impact of one year's reduction in growth to the sustainability of the population is thought to be minimal. Some differences in fledging success and growth of nestlings were observed in the house wren and eastern bluebird, but these differences were not prevalent throughout the length of the study. Due to the inconsistency of results, nestling survival, which was predicted in this study as fledging success and growth, could not be linked to exposure to PCBs.

No data on reproductive measurements in the eastern bluebird or house wren at other PCB-contaminated sites are available to compare. All measures of reproductive success for eastern bluebirds at the Kalamazoo River, except productivity at TB, were similar to those for uncontaminated populations (Pinkowski, 1979). There is little information on the reproductive success of unmanipulated house wren populations, but one population had productivity similar to those of the Kalamazoo River house wren populations (Finch, 1989). Other studies with greater tissue concentrations of PCBs in terrestrial species did not indicate reproductive effects (Bishop et al., 1995; Henning et al., 2002). The observations made in our study, coupled with those made by others at both PCB-contaminated and uncontaminated locations, indicate that it was unlikely that the concentrations of PCBs measured in birds at TB were causing any population-level effects on survival of nestlings.

#### 4.2. Egg measurements

It has been suggested that eggs containing PCBs may exhibit differential dimensions (Fair and Myers, 2002) or mass compared to those of uncontaminated individuals (Fernie et al., 2000). PCBs may contribute to the smaller volume of eggs in ash-throated flycatchers at contaminated sites (Fair and Myers, 2002), but this difference was attributed to interaction with the width parameter in the calculation of egg volume (Pinkowski, 1975). Egg length but not width was evaluated in eggs at the Kalamazoo River, so differences in egg length may not be predictive of PCB exposure. As for measurements of egg weight, the yolks of eggs from PCB-dosed females (34.1 mg PCB/kg, ww in eggs) were found to be greater in mass than that of controls, with greater mass associated with greater lipid content (Fernie et al., 2000). When comparing fresh eggs, the lipid content in house wren eggs from TB was not significantly greater than that of eggs from FC. Sample sizes of fresh eggs of eastern bluebirds were insufficient for analysis. Natural variation in unexposed populations of eastern bluebirds has been described as an egg size gradient ranging from observably smaller eggs (mean length  $\times$  mean width, 19.0  $\times$  15.3 mm) to comparatively larger eggs (22.4  $\times$  17.7 mm). The natural variation inherent in egg size limits the discernment of PCB-induced effects on egg size in this population, but it does not appear that exposure to PCBs had any significant effects on the sizes of eggs.

#### 4.3. Growth measurements

Growth curves generated for each species were similar between sites. Logistic curves gave the best overall fit to the growth data of the Kalamazoo River, which is similar to the results for other locations (Zach and Mayoh, 1982). There are no published growth curves for house wren nestlings, but the relative similarity of curves for birds at TB and FC, over the length of the study, suggests that

growth was not affected by exposure to PCBs. Growth curves for eastern bluebirds at the KRAOC were compared to those for an uncontaminated population (Pinkowski, 1975), and the logistic curve at TB was similar to that at the uncontaminated populations until day 8. From days 8 to 10, the rate of weight increase in Kalamazoo populations decreased relative to that at the uncontaminated site, but weights coincided again by day 12. TB nestling growth during 2001 had the greatest mean nestling mass at each day but the least during 2002. There is no apparent reason for the variation among years, but the inconsistent results among years may be associated with small sample sizes at the site. Nestlings at TB had a curve similar to those at FC, but the mass of nestlings at each day was less. When mass gain for each box over the nestling period of all years was examined, there was no statistically significant difference between TB and FC. The results of a study conducted at another location suggest that availability of insects accounts for 18–51% of the variation, while unexplained variation (error) accounted for a similar proportion (Quinney et al., 1986). Potential variations in error at the Kalamazoo River locations may be associated with variation between individuals, egg quality, or measurement error. Based on visual examination of growth curves and statistical analysis of nestling growth expressed as mass gain per day, no consistent differences in nestling growth were identified when evaluating nests over the entire study period. Although some significant differences between sites existed when years were evaluated separately, the differences were found in only 1 of the 3 years of the study. Thus, it can be concluded that the exposure to PCBs at TB did not affect the rates of growth of either bluebirds or house wrens.

#### 4.4. Correlations between total PCB and TEQ concentration and measurement endpoints

Concentration–effect relationships between the two measures of exposure (PCB or TEQ) and the measures of reproductive performance based on individual nests were examined by correlation analyses—Pearson product-moment correlations and general linear regression models (Table 7). There were no significant correlations ( $P < 0.05$ ) between total concentrations of total PCBs or TEQs and any of the measures of reproductive success for house wrens. There were some significant and negative correlations between concentrations of PCBs and clutch size, hatching success, fledging success and productivity for eastern bluebirds, but only fledging success was correlated with TEQ concentrations even though concentrations of TEQ and PCBs were positively and significantly correlated ( $r^2 = 0.86$ ,  $P < 0.01$ ,  $n = 25$ ).

The trends in correlations were not consistent. Of the four significant negative correlations between concentrations of PCBs, only one, fledging success, was correlated with concentrations of TEQ. For eastern bluebirds, concentrations of TEQs generally described <30% of the

Table 7

Pearson product-moment correlations ( $r^2$ ) with probabilities of exceeding this value by chance alone ( $P$ ) between concentrations of PCBs or TEQ and each of the measurement endpoints for the eastern bluebird and house wren

Species	Exposure	Endpoint	$r^2$	$P >$
Eastern bluebird	Total PCB	<b>Clutch size</b>	(–) <b>0.62</b>	<b>&lt;0.01</b>
		Mean egg weight	(–) 0.48	0.06
		<b>Hatching success</b>	(–) <b>0.28</b>	<b>0.03</b>
		<b>Fledging success</b>	(–) <b>0.93</b>	<b>&lt;0.01</b>
		<b>Productivity</b>	(–) <b>0.33</b>	<b>0.02</b>
	TEQ	Clutch size	(–) 0.09	0.52
		Mean egg weight	(–) 0.27	0.29
		Hatching success	(+) 0.01	0.82
		<b>Fledging success</b>	(+) <b>0.91</b>	<b>0.01</b>
		Productivity	(–) <0.01	0.94
House wren	Total PCB	Clutch size	(+) 0.01	0.62
		Mean egg weight	(+) 0.01	0.65
		Hatching success	(–) 0.13	0.10
		Fledging success	(+) 0.02	0.54
		Productivity	(–) 0.07	0.25
	TEQ	Clutch size	(+) 0.04	0.50
		Mean egg weight	(+) <0.01	0.93
		Hatching success	(–) 0.08	0.32
		Fledging success	(+) 0.03	0.62
		Productivity	(–) 0.04	0.50

Directions of correlations are shown in parentheses. Significant correlations are shown in bold-face.

variability in reproduction, but concentrations of TEQs described 91% of the fledging success and total PCBs explained 28–93% of variability in reproductive parameters. It was determined, after examining the residuals and the scattergram of the data, that these correlations were spurious and were artifacts of the data structure. Although these values seem to show a significant correlation, the sample sizes were small and, because of the difference between concentrations of PCBs at the two locations, a regression based on these values effectively becomes a two-point curve driven by two clusters of data points, which is unreliable for describing relationships. There were no consistent patterns in the relationships between the two measures of exposure and the measures of reproductive performance for any of the species studied. In general, the proportion of the total variance explained by either measure of exposure, total PCBs or TEQs was small.

## 5. Conclusions

Measures of growth and overall productivity were used to evaluate whether adverse population-level effects were occurring in two terrestrial avian species exposed to PCBs at the Kalamazoo River Superfund site. While the values of some measures of reproductive success of house wrens were significantly less at TB than at FC in some years, overall fledging success, which is a predictor of population-level

effects, was greater at TB than at FC. Also during 1 year, growth of each species was less at TB than at FC, but when evaluated over the entire study period, growth of both species was not significantly different between TB and FC. Other studies also examined reproductive effects in terrestrial species due to PCB exposure and indicated no observed effects at concentrations of 18.6 mg PCB/kg, ww (Bishop et al., 1995) to 83.6 mg PCB/kg, ww (Henning et al., 2002) in eggs, which is greater than those contained in eggs at TB (6.3 mg PCB/kg, ww in house wren eggs and 8.3 mg PCB/kg, ww in eastern bluebird eggs). Based on the results of the study reported here and the results of other studies investigating the effects of PCBs on terrestrial passerine birds, it can be concluded that it was unlikely that exposure to PCBs at the Kalamazoo River Superfund site was having adverse effects.

Colocated studies were conducted in conjunction with the present study to quantify the concentration of PCBs in the tissues and diets of Kalamazoo birds and to compare various approaches to estimating risk (Neigh et al., 2006b,c). Concentrations of PCBs were significantly greater in tissues of the eastern bluebird and house wren at TB, which was the more contaminated location, than in tissues of birds at FC, which was the upstream reference area (Neigh et al., 2006b). However, concentrations of PCBs in eggs and nestlings did not exceed a threshold above which effects were expected. Concurrent studies also investigated dietary exposure and determined that total PCBs in the diet of terrestrial species were less than the threshold concentration to cause adverse effects on reproduction (Neigh et al., 2006c). Concentrations of TEQs in the diet were in a range that, based on the most conservative threshold determined from laboratory studies with other species, could cause some adverse effects. However, it was determined that the threshold was not exceeded when toxicity reference values based on a more appropriate field-derived threshold were applied in the risk assessment. The assessment of risk for concentrations in tissues and dietary exposure depend heavily on the threshold of effects and, since there are no studies specifically dealing with the species examined here, there are uncertainties associated with the choice of a threshold concentration, especially with dietary studies.

Even based on an approach to evaluate risk that includes three lines of evidence (tissue concentrations, dietary exposure, and reproduction), it was difficult to definitively determine whether PCBs are eliciting effects on birds at the Kalamazoo River. The results of the studies presented here suggest some reproductive impairment of eastern bluebirds and house wrens at some locations of the Kalamazoo River, but other factors in addition to PCBs, such as cocontaminants, habitat suitability, and prey availability, are likely causes for the effects. Colocated tissue studies at the Kalamazoo River locations (Neigh et al., 2006b) suggested that eastern bluebird eggs contained concentrations of DDT and its isomers near the threshold of effects for raptor species (Elliott and Harris, 2002), and the shells

of house wren eggs at the contaminated location were apparently thinned, which is a known effect of dichlorodiphenyldichloroethylene exposure (Lincer, 1975). Reproductive effects at the Kalamazoo River can not be decisively linked to exposure to PCBs and, with the exception of the potentially conservative estimate of risk based on exposure to non-*ortho* and mono-*ortho*-substituted PCBs, the tissue and dietary lines of evidence arrive at similar conclusions of no unacceptable risk. Based on three lines of evidence, the authors do not believe that terrestrial passerine species are exposed to concentrations of PCBs in contaminated areas of the Kalamazoo River that may contribute to the present or future risk of reproductive dysfunction.

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